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TOXICITY TO SALMONIDS OF THE HERBICIDE GLYPHOSATE (ROUNDUP™) AND ITS SURFACTANT (MON 0818)

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by

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ABSTRACT

In 1984, static fish bioassays were conducted by the Environmental Protection, Pacific Region and Yukon, of Environment Canada to determine the acute toxicity to juvenile salmonids of herbicide Roundup™ and its surfactant MONO-818.

This study indicated that Roundup™ was only moderately toxic to two species of Pacific salmon. The average 96 hr LC_{50} values for the fish were: Coho salmon (42.3 mg/L), and Rainbow trout (32.4 mg/L). However, the surfactant MONO-818 was highly toxic to salmonids, with 96 LC_{50} values of 3.2 and 3.5 mg/L for Rainbow trout and Coho salmon respectively. The stabilisation time of LC_{50} values varied with different fish for Roundup™, but appeared to be 96 hours for the surfactant MON 0818. Analytical results of selected test water samples indicated that the free acid equivalent of glyphosate in Roundup™ was approximately 30 %. Also glyphosate did not degrade to any significant degree during the 96 hour test period.

The implications of these results and their potential short term impact on Pacific salmonid species are discussed.

RESUME

En 1984, des bioessais statiques ont été effectués avec des poissons par le Service de la prtotection de l' environnement, Région du Pacifique, d'Environnement Canada, pour determiner la toxicité aiguë pour les salmonidés juvéniles de l'herbicide Roundup et de son surfactant MON 0818.

Cette étude a indique que le Roundup était modérément toxique pour deux espèces de saumon du Pacifique. Les valeurs de CL_{50} - 96 h moyennes pour les poissons étaient: coho, 42.3 mg/L, et truite arc-en-ciel, 32.4 mg/L. Le surfactant MON 0818 était fortement toxique pour les salmonidés, avec des valeurs de CL_{50} - 96 h de 3.2 et 3.5 mg/L pour la truite arc-en-ciel et le saumon coho, respectivement.

Le temps de stabilisation des valeurs de CL_{50} variait selon les differentes espèces de poissons dans le cas du Roundup, mais il semblait être de 96 heures pour le surfactant MON 0818. Les résultats des analyses d'échantillons d'eau choisis ont indiqué que l'équivalent en acide libre du glyphosate était d'environ 30 % dans le Roundup. En outre, le glyphosate ne se dégradait pas de façon significative au cours de la périodes d'essai de 96 heures.

La signification de ces résultats et leur impact possible à court terme sur les espèces de salmonides du Pacifique sont examinés.

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CONCLUSIONS

1. Roundup™ is moderately toxic to juvenile Pacific salmonids (Coho and Rainbow trout) with an average 96 hr LC_{50} value of 34.9 mg/L (range, 26.7 - 42.3 mg/L).

2. Roundup™ surfactant MON 0818 is about ten times more toxic to juvenile Pacific salmonids (Coho salmon and Rainbow trout) with an average 96 hr LC_{50} value of 3.4 mg/L (range, 3.2 - 3.7 mg/L).

3. Low concentrations of glyphosate (i.e., 0.025 - 0.100 mg/L) in stream water is probably unlikely to have any short term impact on fish.

4 The important components of the surfactant MON 0818 need to be quantified in laboratory and field studies and their long term impact on fish investigated.

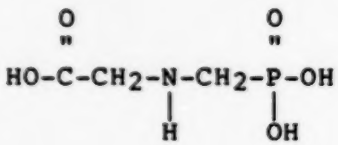
1 INTRODUCTION

Roundup™ is the trade name of a formulated herbicide containing the active ingredient glyphosate. It is used as a selective or non-selective post emergent treatment to remove unwanted vegetation in agriculture, forestry, and rights-of-way. In the coastal forest areas of British Columbia, glyphosate has the potential to replace the phenoxy herbicides, such as 2,4-D, for brown and burn and conifer release programmes.

Most of the fish toxicity information used for the registration of this herbicide in Canada was produced in the U.S.A. There was insufficient information on the acute toxicity of Roundup™ to salmonid species. To assist the regional office of the Environmental Protection in the evaluation of provincial permit referrals for aerial and ground based spray applications of Roundup™, a fish bioassay programme was initiated in 1980 to determine the acute toxicity of Roundup™ and its surfactant to juvenile Pacific salmon.

2 LITERATURE REVIEW

Roundup™ contains the active ingredient glyphosate (N-(phosphono-methyl)-glycine) which has the following structural formula:



Roundup™ contains a polyethoxylated tallow amine surfactant (MON 0818) with several ethylene oxide units, (Monsanto Canada, per comm., 1985). The proportion of glyphosate:surfactant:water in the herbicide Roundup™ formulation is approximately in the ratio of 2:1:3.

Folmar et al (1979) reported that Roundup™ and its surfactant MON 0818 were toxic to juvenile Rainbow trout, with a 96 hr LC^{50} of 1.6 mg/l and 7.4 mg/l respectively. Their study also indicated that Roundup™ and the surfactant were highly toxic to the swim-up fry and fingerlings of the same fish, with a 96 hr LC_{50} value of 2.4 and 1.3 mg/l respectively. The researchers further reported that Roundup™ toxicity to rainbow trout increased with increasing temperatures, i.e., twice as acutely toxic at 17°C than at 7°C. Moreover, Roundup™ was more toxic to rainbow trout in slightly alkaline water (pH = 7.5 - 8.0)

than in slightly acid water (pH = 6.5). A fish avoidance study indicated that rainbow trout did not avoid glyphosate concentrations as high as 10 mg/l, (Folmar, 1979).

The acute toxicity to fish of both the formulated Roundup™ and its active ingredient glyphosate (isopropyl-amine salt) was reported by Tooby (1985). The acute lethal toxicity of Roundup™ varied considerably with different species of fish. The 96 hr LC_{50} values were: Grass carp yearlings (15 mg/l), fingerlings of Fathead minnows (2.3 mg/l), Bluegill sunfish (5.0-24 mg/l), Channel catfish (13-19.5 mg/l), and Rainbow trout (8.3-54.8 mg/l). Tooby (1985) also reported the acute lethal toxicity of the active ingredient glyphosate to several fish. The 96 hr LC_{50} values for the fingerlings of these fish were: Fathead minnow (97 mg/l), Channel catfish (130 mg/l), Bluegill sunfish (>24-140 mg/l), and Rainbow trout (50-97mg/l). These data seemed to indicate that glyphosate was relatively less toxic to fish than the formulated Roundup™. Except for Rainbow trout, most of the fish species were subtropical, and there was a lack of information on the toxicity of both Roundup™ and its active ingredient to Pacific salmon.

3 MATERIALS AND METHODS

3.1 Bioassay Laboratory

The study was conducted at the Environmental Protection Aquatic Toxicity Laboratory, 1801 Welch Street, North Vancouver, British Columbia.

3.2 Formulation Tested

Both the formulated Roundup™ and its surfactant (MONO 818) were tested for acute lethal toxicity to a number of juvenile Pacific salmon species.

The amount of chemicals required to produce the test concentrations was prepared on a volume/volume basis in clean volumetric flasks and introduced to the test vessels as described in section 3.5.

3.3 Test Fish

Underyearling Rainbow trout (Salmo gairdneri) and Coho salmon (Oncorhynchus kisutch) were used as the test fish. Rainbow trout were obtained as fry from the B.C. Provincial Hatcheries at Summerland (Spring) and at Abbotsford (Fall), while coho salmon fry were obtained from the Inch Creek Hatchery, Fraser Valley, (Spring). Prior to testing, these fish were acclimated to laboratory conditions for a minimum period of one month. They were reared in fiberglass

tanks at ambient water temperature of 6°C (range, 4.5°C-8°C) and were fed a maintenance diet of Oregon moist pellets. Fish under test were not fed. The photo-period for holding, and during the tests was 14 hours of light (vita-lite) and 10 hours of darkness, with two 15 minute twilight periods.

3.4 Fish Culturing Conditions

Dechlorinated Vancouver city tap water was used for fish culturing. It also served as a diluent water for the test. The water quality parameters were: temperature, 4.5 - 8°C; hardness, 10.2 $\pm$ 5 mg/l $CaCO_3$; conductivity, 10 $\pm$ 5 uMHOS; pH, 6.1 $\pm$ 1; alkalinity, 2.5 $\pm$ 0.5 mg/L $CaCO_3$. These measurements represent average values over a four month period. There was little variability in culturing conditions between each bioassay test.

3.5 Fish Bioassay Procedure

Fish bioassays were based on protocols from Environment Canada (1980) and the British Columbia Ministry of Environment Bioassay Task Force (1982). Glass aquaria (approximately 45 L capacity, measuring 27 h x 21 w x 54 cm) were used as the test vessels. A maximum volume of 35 litres of dechlorinated city tap water was used in each test vessel. Oil free compressed air was bubbled through the water column at a rate of 200 ml/min during the test and for

a period of 30 minutes prior to the introduction of the herbicides. The required volume of test chemical, prepared as described in section 3.2, was added into each test vessel, and allowed to mix for 5-10 minutes to disperse the herbicides prior to the introduction of test fish.

Ten fish were placed in each test vessel. Observations of fish mortality were recorded at 24, 48, 72, and 96 hr after fish introduction. A fish was considered dead when all movement ceased and no further responses could be obtained by prodding or moving the fish. Fish found dead during the test were removed at each observation.

The results of cumulative fish mortality recorded over four days were used for the calculation of LC50 values using a computer "Lethal" programme developed by Charles Stephans (6201 Cougdon Blvd. Duluth, Minn. 55804).

3.6 Residue Sampling Procedure

Water samples were collected in 450 ml amber glass bottles to ascertain the actual concentration of glyphosate in the water of selected test vessels. All bottles were rinsed with redistilled acetone and petroleum ether, and heated to 300°C for 12 hours prior to use. A piece of solvent washed and heat treated aluminium foil was used to line the opening of each bottle before capping to prevent sample contamination. Water samples were collected at 0 hr,

24 hr, 48 hr, 72 hr and 96 hr.

3.7 Residue Analysis

All residue analyses were conducted by the British Columbia Ministry of Environment Laboratory. Water samples were derivatized and cleaned up prior to ion supression High Performance Liquid Chromatography (HPLC). The analysis was performed on a Hewlett Packard 1084B HPLC with an ultrasphere ODS 5 u column.

The detection limit of glyphosate and its main metabolite amino-methyl phosphonic acid (AMPA) was 0.005 mg/L. The average recovery rate for both materials was greater than 95%.

4 RESULTS AND DISCUSSIONS

4.1 Water quality (diluent water) and fish condition

The water quality of the diluent water for each of the test vessels at the Environmental Protection Aquatic Toxicity Laboratory was similar in all the bioassay tests, (Table 1). Hardness as the calcium carbonate equivalent averaged 4.8 mg/L (range, 4.1-5.3), and the pH and water temperature remained uniform at 6.4 (range, 6.3-6.6) and 14.9°C (range, 14.5-15.0) respectively. The test vessels were aerated at the rate of 200 ml per minute. Meehan et al. (1974) showed that aeration of fish tank water is essential to prevent premature fish mortality, due in part to the depletion of dissolved oxygen. Fish loading density, length, and weight averaged 0.23 gm/L (range, 0.14-0.50), 3.95 cm (range, 3.4-6.0), and 0.6 gm (range, 0.3-2.2 gm) respectively.

4.2 Toxicity to Pacific Salmonids

The results of the fish bioassay tests (Table 1) indicated that Roundup™ was moderately toxic to two species of Pacific salmonids, i.e., Rainbow trout and coho salmon, with an average 96hr LC_{50} value of 34.9 mg/L (range, 26.7-42.3 mg/L). In two of the tests, the LC_{50} values appeared to have stabilised after 24 hours, while two other tests

TABLE 1 TOXICITY TO PACIFIC SALMONIDS OF ROUNDUP™

TEST FISH	TEMP. (°C)	pH	HARDNESS mg/l $CaCO_3$	LC50 (mg/l)			
				24h	48h	72h	96h
Rainbow trout (3.9cm,0.6gm)	14.5	6.4	4.1	42.3	39.9	36.4	26.7
Rainbow trout (6.0cm,2.2gm)	15.0	6.6	4.5	45.7	45.7	45.7	42.3
Rainbow trout (3.4cm,0.3gm)	15.0	6.3	5.3	63.9	42.3	42.3	28.1
Coho salmon (3.5cm,0.3gm)	15.0	6.3	5.3	42.3	42.3	42.3	42.3

indicated that the LC_{50} values decreased with time. It was not possible to verify this trend as the tests were terminated after four days.

The 96 hr LC_{50} values for Rainbow trout and Coho salmon were not significantly different from the results of tests conducted with Sockeye salmon at the Department of Fisheries and Oceans Research Laboratory (Cultus Lake), despite the differences in test water conditions (Servizi et al.,1987). The water used for our bioassay was fairly soft, slightly acidic and much higher in temperature when compared to the water conditions of Servizi et al. (1987). These results differ from Folmar et al. (1979) which indicated that Roundup™ is more toxic to Rainbow trout at higher water temperatures and under slightly alkaline conditions. Folmar et al. (1979) also reported that Roundup™ is highly toxic to Rainbow trout swim-up fry and fingerlings with 96 hr LC_{50} values of 2.4 mg/L and 1.3 mg/L respectively. However, the present study as well as Servizi et al. (1987) showed that Roundup was much less toxic to Rainbow fry.

Table 2 shows the toxicity results of Roundup™ surfactant MON 0818 to two species of salmonids. MON 0818 is highly toxic to juvenile Rainbow trout and Coho salmon, with an average 96 hr LC_{50} value of 3.4 gm/L (range, 3.2 - 3.5 mg/L). Servizi et al. (1987) shows that MON 0818

TABLE 2 TOXICITY OF ROUNDUP™ SURFACTANT MON 0818 TO PACIFIC SALMONIDS

TEST FISH	TEMP. (°C)	pH	HARDNESS mg/l $CaCO_3$	LC50(mg/l)			
				24h	48h	72h	96h
Rainbow trout (3.4cm,0.3gm)	14	6.2	5.3	4.1	3.2	3.2	3.2
Coho salmon (3.5cm,0.3gm)	14	6.2	5.3	5.0	4.5	4.5	3.5

is also highly toxic to Sockeye salmon with a 96 hr LC_{50} value of 2.6 mg/L. MON 0818 is more toxic to Pacific salmonids than Roundup™ by a factor of ten, which concurs with the findings of Folmar et al. (1979).

The nominal concentration of Roundup™ and glyphosate, and the measured concentration of glyphosate in the test water samples are shown in Table 3. The analysis of water samples for the nominal concentration of glyphosate confirmed that Roundup™ contains approximately 30 % glyphosate free acid. It also showed that glyphosate did not degrade significantly during the 96 hour test period. In the study conducted by Folmar et al.(1979), the water samples were not analysed to determine the actual concentration of glyphosate. Therefore, it was not possible to identify the precise concentration of this herbicide in Folmer's test series that indicated that Roundup was highly toxic to both Rainbow trout swim-up fry and fingerlings.

4.3 Implications of Short Term Toxicity of Roundup and its Surfactant on Fish and Aquatic Organisms

The potential short term toxicity Roundup to fish and aquatic organisms could be evaluated as a consequence of the known concentrations of this herbicide reaching the stream water. One monitoring study found that the highest level of glyphosate reaching a stream not protected by a

TABLE 3 NOMINAL AND MEASURED CONCENTRATION OF GLYPHOSATE AND AMPA* IN SELECTED BIOASSAY TEST WATER SAMPLES

TEST FISH	NOMINAL CONCENTRATION IN TEST WATER SAMPLES (gm/l)		MEASURED CONCENTRATION OF GLYPHOSATE (gm/l) IN TEST WATER SAMPLES				
	ROUNDUP™	GLYPHOSATE	0h	24h	48h	72h	96h
Rainbow trout	58	18	18	19	18	19	19
Coho salmon	32	10	11	11	10	11	11

* The measured concentration of AMPA, the main metabolite of glyphosate, was less than 2 mg/l in all the water samples.

buffer zone was 0.100 mg/L (Wan, 1986). This concentration is equivalent to a concentration of 0.327 mg/L Roundup™ in water, and was a transient phenomenon. The results of the present static fish bioassay tests suggest that a transient level of 0.327 mg/L Roundup™ in stream water will not cause fish mortality, because the 96 hr LC_{50} values of three Pacific salmonids to this herbicide averaged 27.7 gm/L. Even the worse case 96 hr LC_{50} results reported by Folmar et al (1979) were 1.3 mg/L for Rainbow fingerlings, and this value was approximately 4 times higher than the transient level of Roundup™ found in the stream.

In the same study by Wan (1986), the maximum transient concentration of glyphosate found in streams protected by a 60 meter buffer zone was 0.025 mg/L, which was equivalent to 0.082 mg/L Roundup™ in water. This amount of transient Roundup™ was too low to have any significant short term impact on fish.

Folmar et al.(1979) reported that the 48 hr LC_{50} value for Roundup™ to Daphnia was 3.0 mg/L (range, 2.6-3.4) at 22°C; while Servizi et al (1987), found that the 96 hr LC_{50} of this herbicide to the same organism was 25.5 mg/L at 21°C. Despite the differences in measured acute toxicity, a concentration of 0.327 mg/L Roundup™ in streams is unlikely to have any significant short term impact on Daphnia.

In summary, this study and others indicate that the surfactant MON 0818 plays a major role in the acute toxicity of Roundup™ herbicide. Glyphosate alone is relatively much less toxic to fish. It is suggested that future studies should attempt to quantify important components of the surfactant in test water samples from both laboratory and field studies, and determine its long term impact on Pacific salmonids.

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